Molecular Biology

RAPID ASSAY FOR DETERMINATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) PHENOTYPE

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HIV-1 requires a chemokine receptor, mainly CCR5 or CXCR4, in addition to the main receptor CD4, for successful infection of cells. The majority of HIV-1 strains isolated from early-stage acquired immunodeficiency syndrome (AIDS) patients exhibit phenotype R5, using CCR5 as a co-receptor. However, the emergence of X4 HIV-1 strains utilizing CXCR4 is often associated with accelerated disease progression. The standard test to assess HIV-1 phenotype, the MT-2 syncytia assay, requires prolonged virus propagation, and favors patient virus strains that replicate well in culture. The phenotypes of growth kinetics, cytopathicity, and co-receptor usage are determined by the variable HIV-1 gp120 regions V1-V3, suggesting that rapid molecular tests could be developed. With the development of anti-HIV drugs targeting CXCR4 gp120 interactions, a rapid screening test is essential to identify patients that would benefit from anti-CXCR4 therapy.

We have developed a novel, rapid phenotyping strategy, in which pseudotype virus is generated by co-transfection of HIV-1 envelope (*env*) genes, with a full-length proviral clone defective in *env* and containing firefly luciferase *(luc*) in place of the accessory gene *nef*. Pseudotype virus is used to infect cell lines engineered to express CD4 and either CCR5 or CXCR4. Expression of the *luc* gene in infected cells is used to quantify virus entry by a luminometric assay. Currently, we are working to optimize protocols for amplifying *env* sequences from patient blood by RT-PCR, which are used to generate patient-specific HIV-Luc pseudotype virus. The presence of X4 virus strains is shown by ability to infect cells expressing CD4 and CXCR4, but not CD4 and CCR5. In contrast to previous HIV-1 phenotype tests, this assay is rapid, highly sensitive, and provides a quantitative entry report.

Supported by The University of Texas Medical Branch Summer Undergraduate Research Program and NIH grant #RO1-AI-462510-02.